THE EFFECT OF ELECTRIC CURRENT ON THE RELATIVE VISCOSITY OF SEA-URCHIN EGG PROTOPLASM[1]

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Becquerel (1837) was the first investigator to study the action of electricity on protoplasm of single cells. [The greater part of the literature treating of the effect of electric current on cyclosis may be obtained from Ewart (1903).] The conclusion to be drawn from the literature is that electric current, depending on the current density employed, produces a progressive decrease in, followed ultimately by cessation of, cyclosis providing the current flows for a sufficient interval of time. However, Velten (1876), Ewart (1903) and Koketsu (1923) observed an initial increase prior to the characteristic progressive slowing of cyclosis. The results obtained by Brücke (1862) on human leucocytes, Chifflot and Gautier (1905) on *Cosmarium,* Bayliss (1920) on *Tradescantia* and *Amoeba* by the Brownian movement method and Bersa and Weber (1922) on *Phaseolus* by the centrifuge method are in essential agreement, i.e., electric current produces an increase in protoplasmic viscosity.

This investigation was undertaken to continue and extend the study of electric current as a stimulating agent to some type of protoplasm other than that of the protozoan cells *Amoeba dubia* and *A. proteus* already studied (1937).[4] It is of interest to know whether the protoplasm of such distantly related biological groups, e.g., *Amoeba* and the unfertilized eggs of *Arbacia,* responds to this stimulating agent in a comparable manner.

Material and Methods

The experiments were performed on the unfertilized eggs of the sea-urchin, *Arbacia punctulata.* The eggs were treated according to method " 3 " as described by Just (1928). Eggs were shed in about

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[4] Our own studies on amoebae are now nearing completion and a detailed report will appear shortly.

250 cc. of sea water and washed once in an approximately equal volume of the medium.

The eggs were subjected to either direct or alternating electric current, according to the experiment in question, in a celluloid trough, the sides of which were perfectly milled and thus parallel to the lines of current flow. The current was applied through a $Zn/ZnSO_4/$ sea-water-in-agar system, which, in turn, was in circuit with a reversing switch, rheostat and milliammeter. The agar bridges were cut to fill the ends of the trough completely and were finally sealed in place by means of hot agar. The available electrode surface, i.e., the cross-sectional area of the available medium in the trough, was 40 mm^2. The source of the electric current was the regular service line (110 volts) running into the laboratory.

The protoplasmic viscosity was determined by the centrifuge method. The handle of an Emerson hand centrifuge, when turned at the rate of one revolution per two seconds, developed a centrifugal force of 2,531 times gravity, after allowance was made for the depth to which the eggs settled in the centrifuge tube when cushioned on an isosmotic (0.73 m.) sucrose solution. The eggs were centrifuged until 80 per cent, or more, showed a fine hyaline band 2/15 the diameter of the egg appearing between the oil cap and the yolk granules. (This fraction was equal to one division of the arbitrary scale of the ocular micrometer employed in these experiments.) The time in seconds necessary to move the yolk granules the specified distance, and thus show the requisite width of the hyaline band at the centripetal pole of the egg, is designated the "centrifuging value." This is the end-point to which all experimental centrifugalizations are referred.

The experiments were conducted in the following manner. Each batch, i.e., eggs from one female, was tested for 'normalcy.' A batch of eggs was declared 'normal' if, on sampling, 95 per cent or more showed membrane elevation after insemination and 80 per cent or more, showed the desired width of the hyaline area when centrifuged for 60 seconds at room temperature (19°–24° C.). These conditions prevailing, approximately uniform quantities of eggs were placed in the stimulating trough and while the eggs were more or less suspended, an electric current of known intensity and duration of flow was admitted. At the cessation of the current the eggs were immediately pipetted into the centrifuge tube, which contained a known depth of isosmotic sucrose solution, and were centrifuged respectively for various known periods of time. The lowest value to which the interval of time elapsing between the cessation of the application of the stimulating agent and incipient centrifugalization could be reduced was 7 seconds. There was no

apparent difference between this and the 10-second interval which was used throughout these experiments unless otherwise stated.

The points of curves *A* and *B* (Fig. 1) which represent centrifuging values plotted as functions of the time of exposure to the electric current in question were obtained in the following manner. After preliminary tests in which various constant current densities were studied, when applied for varying intervals of time, it was apparent that a current density [5] of 0.005 amperes/mm.^{2} served best to illustrate the results and this density was employed throughout these experiments.

On the basis of the above procedure the curves were developed as

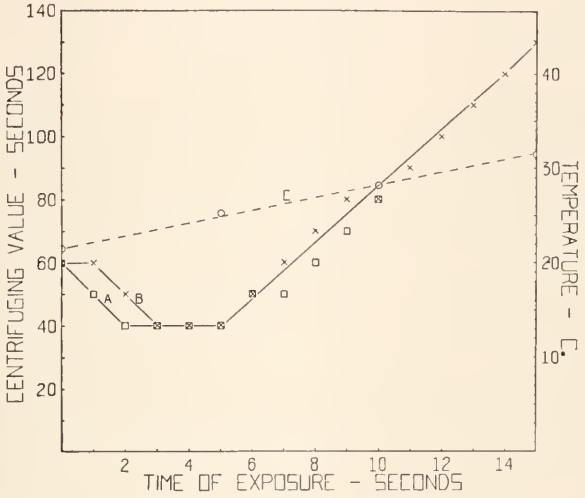

FIG. 1. Centrifuging time in seconds (viscosity) of *Arbacia* egg protoplasm vs. time of exposure in seconds to alternating (curve *A*) and direct (curve *B*) electric current. Curve *C* represents the rate of thermal increase to which the stimulating trough is subjected vs. time of exposure to either type of current. Current density = 0.005 amperes/mm.^{2}; pH 8.2; room temperature = 21.5° C.

follows. In the early experiments several batches of eggs were necessary in order to work out a complete curve. With more experience one batch of eggs sufficed for the determination of the points for any one curve. Twelve curves for direct (*B*) and ten curves for alternating (*A*) current were thus worked out. Finally, with these data as a basis, all points for both alternating and direct current curves were obtained by employing one batch of eggs. This procedure was repeated for three different batches of eggs and thus assured relatively constant conditions of material, temperature (21.5° C.), etc. Hence fewer experiments were performed in the latter instance but these data were substantiated by the

[5] In a preliminary note (1938) amperes/cm.^{2} should have read amperes/mm.^{2}.

more detailed experiments performed during the early part of the work. Under the conditions of the experiments no point on the curves deviated more than ten arbitrary units in centrifuging value in repetitive tests.

Eggs were not used for experimental purposes after being shed longer than three hours. The viscosity of the main protoplasmic mass, as determined by the method here employed, does not undergo any appreciable change during this three-hour period (Goldforb, 1935; Angerer, 1937).

There was no observable difference when experimentally treated eggs were compared with their controls for membrane elevation and cleavage.

Results

Direct Current

When sea-urchin eggs are subjected to a current density of 0.005 amperes/mm.^{2} flowing for varying known intervals of time, there is, in the majority of experiments, no observable change in the centrifuging value (curve B) after one second of continuous exposure.[6] On continuous application of the current for two seconds, there is, in all experiments, a decrease in the centrifuging value; while with three seconds of continuous exposure the viscosity decreases from the control centrifuging value of 60 to a minimum value of 40 arbitrary units, i.e., a decrement of 33 per cent in three seconds. There is no further change in viscosity for the next four or five seconds, respectively, of continuous exposure to the current.[7] However, if the current is allowed to flow for six seconds, the centrifuging value increases from a transient minimum value until after seven seconds of constant exposure the centrifuging value is identical with that of the control. There is a progressive increase on further exposure, so that after fifteen seconds, when these experiments were discontinued, the centrifuging value had increased 225 per cent above the previous minimum value.

Alternating Current

When the centrifuging values are plotted as functions of the time of exposure in seconds (curve A) to alternating current of 0.005 amperes/

[6] In a few experiments the viscosity at the end of this period of time was found to show a slight decrease which was never greater than a centrifuging value of ten seconds.

[7] Occasionally eggs, after exposure to electric current, showed a tendency for the intracellular granules to stick in the cortical area. This condition, though infrequent, was confined, more particularly, to eggs from certain females. Though these eggs were discarded in the final count, since the behavior of the main mass of the protoplasm was of chief interest, their number was not of such magnitude as to affect appreciably the results.

mm.[2] there is, in all experiments, after one second of constant current flow a decrease in the viscosity of the protoplasm as measured by the centrifuging value. This value is decreased further when the eggs are exposed to the current for two seconds, while after three seconds the centrifuging value undergoes no further decrease but levels at the new minimum value which is 67 per cent of the control. This minimum value is maintained after continuous exposure for three, four and five seconds, respectively.[7] Continuing the exposure to the stimulating agent further, there is, after five seconds, a perceptible increase in the viscosity value. Thereafter, with each successive second of exposure to the electric current a progressive increase is noted in the centrifuging value. The increment is of the same value as that recorded for direct current (curve *B*), namely 225 per cent in ten seconds. The results recorded (curve *A*) were discontinued in this experiment after ten seconds exposure to alternating current because of the expiration of the time limit set upon the use of shed eggs.

It was of interest, in view of the high resistance at the sea water-agar interface, to determine the thermal change occurring within the stimulating trough as a result of the passage of an electric current of specified density. The main coördinates of the broken-line curve *C* (labeled at the right and lower sides of Fig. 1) represent temperature as a function of time during which the eggs contained in the trough are exposed to the thermal effect induced by passage of the electric current. The temperature data were obtained by a specially constructed, direct reading thermometer, the bulb of which was of such size as to be submerged completely when immersed in the stimulating trough.

That thermal effects of the magnitude present during the course of these experiments have no observable effect on the centrifuging value is shown on immersing eggs in sea water which has been warmed previously to 32° C. When eggs are exposed to this temperature for greater intervals of time (e.g., 20 seconds) than that to which they are subjected during the course of these experiments and are simultaneously centrifuged with eggs serving as controls, it is found that the centrifuging values are identical. Heilbrunn (1924), though not primarily interested in this phase of the question, states in the protocol (p. 192) of his experiments on heat coagulation in sea-urchin eggs that after five minutes exposure to 32.9° C. the heat-treated eggs were found to show the same width of the hyaline area as the controls.

Experiments were conducted to test whether varying the quantity of eggs suspended in unit volume of sea water in the specified electric field had any tendency to alter the shape of the electric current-viscosity curves. Batches of eggs were allowed to settle under the influence of

gravity in a four-inch finger bowl and minimum amounts of sea water plus the relatively concentrated eggs were picked up by means of a regular medicine dropper. Various points on the electric current-viscosity curves were investigated using one, ten and twenty drops respectively of the egg suspension in unit volume of bathing medium. In the various concentrations of eggs employed there is no observable difference in the centrifuging values other than that which is within the range of experimental error.

Discussion

When either direct or alternating electric current of the intensity employed in these experiments is used as a stimulating agent, there is initially a transient decrease followed by a progressive increase in the viscosity of the protoplasm of sea-urchin eggs. An ultimate increase in the centrifuging value is in line with the literature (see introduction); though no lucid evidence is to be found in favor of a transitory decrease in viscosity prior to the ultimate increase.

In view of Heilbronn's (1914) results on the attempt to correlate cyclosis in terms of viscosity data, it is justifiable to consider only, at the present time, data as obtained from the methods of Brownian movement and centrifugalization. Brücke (1862), Kühne (1864), Chifflot and Gautier (1905), and Bayliss (1920) have observed a decrease or stoppage of Brownian movement on passage of an electric current through the cell. These data, insofar as a definite statement as to the experimental procedure is given, were obtained during the actual passage of electric current through the material in question and not immediately thereafter as in the experiments here reported. There may be some criticism of studying Brownian movement during the actual passage of the current since cytoplasmic granules undergoing electrophoresis lose their characteristic trembling movements (unpublished results). Mast (1931) should be consulted in this connection. Bersa and Weber (1922), using the centrifuge method, observed an increase in the viscosity of the protoplasm of *Phaseolus* on the passage of electric current for relatively long periods of time. It would be of interest to ascertain data for shorter intervals of time.

There is no difference in the results whether one employs alternating or direct current providing identical current densities are employed (compare curves *A* and *B*). Alternating current tends to be more effective initially owing, apparently, to the greater shearing effect produced by the protoplasmic granules suspended in an oscillating electric field which would tend to break down the protoplasmic structure. This effect may be reenforced by the apparently thixotropic character of

protoplasm. For a review of the literature on thixotropy in living cells see Angerer (1936).

The question arises as to the congruity of applying a stimulating agent for the duration of a few seconds while a minimum of 50 seconds is required for obtaining the viscosity determination. When varying intervals of time are permitted to elapse from the cessation of the electric current to incipient centrifugalization, the results obtained are found not to vary for at least two minutes.

The data presented here are in accord with the known facts concerning the action of certain stimulating agents on sea-urchin egg protoplasm. Heilbrunn and Young (1930) and Angerer (1937),[8] employing respectively ultra-violet radiations and mechanical agitation, found a transitory liquefaction prior to an ultimate increase in viscosity. Similar results were obtained for ultra-violet radiations (Heilbrunn and Daugherty, 1933), mechanical agitation, electric current and suddenly applied thermal increments (Angerer, 1936, 1938, 1940) on *Amoeba* protoplasm. To explain their results, Heilbrunn and Daugherty (1933) proposed a theory in terms of colloid chemical changes in protoplasm; for a detailed review of this theory one is referred to Chapter 37 of Heilbrunn's book (1937).

Summary

1. The centrifuge method was used to determine the viscosity of sea-urchin egg protoplasm after exposure to either direct or alternating electric current to a current density of 0.005 amperes/mm.^{2} for various known intervals of time.

2. There is, on exposing eggs to either direct (curve *B*) or alternating (curve *A*) current, a transient decrease followed ultimately by a progressive increase in the centrifuging value (Fig. 1).

3. Since the data for the action of electric current, as employed in these experiments, show a striking similarity to those results as obtained by the use of certain other stimulating agents on *Amoeba* and *Arbacia* egg protoplasm, it is suggested that the mechanism offered by Heilbrunn (1937) may be applicable here.

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[8] Footnote p. 340.

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